Some Airborne Algae from North Central Florida

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Although airborne algae have been collected and studied in several widely scattered locations in the United States (Luty and Hoshaw, 1967), there are no records previous to this study of airborne algae from Florida. The purpose of this paper is to report the algae collected from the atmosphere over the University of Florida campus from January to August of 1969. It is part of the M.S. thesis of the senior author submitted to the Graduate School of the University of Florida.

Materials and Methods

The culture medium used in this investigation was Bristol's medium (Bold and Parker, 1962) enriched by adding 2 drops of a 3 per cent solution of $NaSiO_3$ to each liter. This medium is widely used for the culture of soil algae, soil being the source of most airborne algae.

All plates exposed to the atmosphere, receiving the rain water or coated collector rods from the Rotorod sampled were cultured under fluorescent lamps (Plantgro, Westinghouse) with an intensity of 400 foot-candles and with a 16-8 hr. light-dark cycle. The temperature in the culture room was kept at $25\pm2°C$. Viable algae developed into macroscopically-visable colonies after 2 to 4 weeks. Each of these colonies was transferred to a tube containing sterile Bristol's nutrient agar and kept as a stock culture under the lights for subsequent study. All transfers were made in a sterile transfer chamber.

Sampling Methods

Hand-held agar plate. Agar plates containing the modified Bristol's medium were exposed from a moving automobile, from January 1969 to July 1969, according to methods of Brown, Larson and Bold (1964). The plates were held by hand and in a vertical position for 20 seconds to 5 minutes; the speed of the vehicle was 25 mph. After exposure, the plates were placed under the fluorescent lights in the culture room.

Rain water. Rain water was collected in a sterile 125-ml Erlenmeyer flask fitted with a small funnel. The flask and funnel assembly was placed 10 feet above the ground at the time that the rain started falling. Immediately after collecting the water, 2 ml portions were transferred by a sterile 5-ml disposable pipette to agar plates containing sterile Bristol's medium. The water was dispersed over the surface of the agar by swirling the dish on a flat surface, and the plate was then placed under the fluorescent lights in the culture room.

Rottorod sampled. This air sampling apparatus (Metronics Associates, Palo Alto, California, Model 65A) is capable of sampling particles from 5 to 100 microns in size and has a filtering rate of 60 liters per minute. The particles are impacted on the leading surface of the clear plastic rotating collector rods coated with a thin layer of silicone compound (General Electric G-697). Immediately after each 10 minute sampling, the collector rods were taken from the metal holder by sterile forceps and placed in a sterile transit vial. In the laboratory, the exposed rods were taken from the transit vial and streaked on the surface of an agar plate containing the sterile nutrient medium. This procedure was conducted in a sterile transfer chamber. Once streaked, the rods were left on the agar with the silicone-coated surface down so that any viable cells left on the rods would develop into colonies (Brown, Larson and Bold, 1964). The plates were then placed under the fluorescent lights in the culture room. The silicone compound was chosen to coat the rods since it strongly retains the impinged particles.

Ten-minute samples were taken with the Rotorod sampler from the 4 stations listed below located on the campus of the University of Florida, from June 25, to August 19, 1969. Two of these stations were located 12 feet above the ground in open fields and 2 stations were located 12 feet above the roof tops of 2 different buildings. The buildings were chosen for their height and for the lack of algal growth on the roof, as far as the authors could observe.

Station 1. Top of the press box, Florida Field, approximately 90 feet high.

Station 2. Observation platform, Space Sciences Center, approximately 60 feet high.

Station 3. Parking lot (paved), corner of Center Drive and Museum Road.

Station 4. Parade review grounds.

Sedimentation method. Sterile agar plates were exposed from 10 p.m. to 9 a.m. on 5 different occasions, 10 feet above the ground in front of McCarty Hall. These samples were taken on clear and relatively calm nights during the month of July, 1969. After exposure, the plates were placed under the fluorescent lights in the culture room.

The morphology of the algae was studied by making fresh mounts from cultures grown on agar or in the liquid medium. The morphology of the chromatophore of the green algae, an important criterion established by Starr (1955) in the taxonomy of the Chlorococcales, was studied by using a blue light filter as proposed by Friedmann (1966). The presence of a gelatinous matrix was demonstrated by using a solution of methlene blue. Sudan IV was used to determine the presence of fat or oil within the cells, and solutions of I_2-KI were used to demonstrate the presence of starch. The number and position of the nuclei was determined by the acetocarmine technique according to the method of Cave and Pocock (1951).

In order to promote the formation of zoospores in the zoospore-producing genera of the Chlorococcales, the following technique proved successful. A culture was started from a stock culture by streaking the algae on fresh agar plates containing sterile nutrient medium. When the culture was growing vigorously, the plate was flooded with sterile liquid nutrient medium or sterile distilled water, and the culture was then placed under the fluorescent lights for 24 hours. Microscopic observations were made the following morning.

All algal isolates were surveyed, and only Chlorophyta and Cyanophyta were present. All the blue-green isolates were identified; Dr. Francis Drouet determined a number of the species in the Oscillatoriaceae. Although all the filamentous species of the Chlorophyta were identified, only 80 of the coccoid Chlorophyta were identified because of time limitation; all of these belonged to the orders Chlorococcales or Chlorosphaerales. In species determination of the coccoid members of the Chlorophyta, Starr (1955), and Brown, Larson and Bold (1964) emphasized the necessity of long periods of observations of unialgal cultures before successfully disposing of the taxonomy of most of these genera.

RESULTS

Hand-held plates. Seventy three samples were taken by this method, 39 of which contained viable algae, yielding 193 colonies. The usual number of impactions per plate ranged from 4 to 6; the speed of 25 mph was found to yield the most impactions. The number of impactions was slightly higher during the spring and summer months than during the winter. Twenty-five species were recovered by this method of sampling. The most common species thus obtained was the blue-green *Schizothrix calciola*, with 23 impactions.

Rain water. Thirty six plates were inoculated with rain water, 12 of which developed algal colonies after 2 to 3 weeks under the fluorescent lights. Twenty unialgal cultures were obtained. *Schizothrix calcicola* was the only blue-green recovered from rain water. The most common species of the green algae thus obtained were *Chlorella vulgaris, C. saccharophila* and an unidentified species of *Oocystis.*

Sedimentation method. Five agar plates were exposed, 4 of which were positive with 31 colonies isolated into unialgal cultures. Only by this method was *Scytonema ocellatum* recovered. Several members of the Chlorophyta were also obtained by sedimentation.

Rotorod Sampler. Sixty samples were taken by this method, 42 of which were positive, yielding 189 isolates when grown under the fluorescent lights. Thirty-four samples were taken during the morning hours, between 9 a.m. and 12 noon. Twenty-one of these samples were positive, yielding 53 colonies which were isolated into unialgal cultures. The morning samples yielded an average of 2.6 impactions per cubic meter of air sampled. Twenty six samples were taken in the afternoon hours, between 2 p.m. and 6 p.m. during the sampling period. In contrast with the morning samples, all but 2 of the afternoon samples developed algal colonies when placed under the fluorescent lights. A total of 136 colonies were isolated into unialgal cultures from the afternoon sampling, yielding an average of 8.7 impactions per cubic meter of air. In the counting of the number of impactions, the assumption was made that each impaction was from a single cell which produced a single colony when the collector rod was streaked on the agar surface.

The largest number of impactions obtained by the Rotorod sampler was recovered during the dry part of the sampling period, when the total amount of precipitation recorded was 0.10 inches of rain.

TABLE 1
Number of algal isolates obtained by 4 sampling methods

Species	Hand Held Plates	Rotorod Sampler	Rain Water	Sedimentation	Total
CHLOROPHYTA					
Ankistrodesmus sp.			1	1	2
Chlamydomonas globosa Snow	1	6			7
Chlamydomonas sp.	1			1	2
Chlorella luteoviridis Chodat	1	1	1		3
Chlorella saccharophila (Kruger) Migula	3	9	3		15
Chlorella vulgaris Beijerinck	3	3	4		10
Chlorococcum ellipsoideum Deason & Bold		2			2
Chlorococcum scabellum Deason & Bold				1	1
Chlorosarcina sp.		2			2
Chlorosarcinopsis aggregata Arce & Bold		1			1
Chlorosarcinopsis dissociata Herndon	1				1
Chlorosarcinopsis minor Herdon		1			1
Chlorosarcinopsis sp.	1				1
Hormidium flaccidium A. Braun	1		1		2
Nannochloris bacillaris Naumann	1	1			2
Neochloris sp.	1				1
Oocystis polymorpha Grover & Bold	4	5	2		11
Oocystis sp.		1			1
Scenedesmus quadricauda	4	1			5
Oocystis sp.'			3		3
Spongiochloris incrassata Chantanachat & Bold				1	1
Stichococcus bacillaris Näg.	3				3
Stichococcus mirabilis Lagerh.	3				3

The temperature during the sampling period ranged from 20°C at night to 37°C during the day. The wind direction seemed to have little influence on the number of impactions. However, a wind speed of 15 mph yielded samples with the largest number of impactions. Partly cloudy skies favored higher numbers of impactions than did clear skies. The relative humidity during the sampling period ranged from 40 per cent to 100 per cent on several occasions during the month of July.

TABLE 2
Number of algal isolates obtained by 4 sampling methods

Species	Hand Held Plates	Rotorod Sampler	Rain Water	Sedimentation	Total
CYANOPHYTA					
Anabaena flos-aquae (Lyngb.) Breb.	1				1
Anabaena variabilis Kützing	5	1			6
Anabaena sp.		2			2
Calothrix parietina (Nägeli) Thuret	4	1			5
Fischerella ambigua (Näg.) Gom.	1	1			2
Oscillatoria formosa Bory.	1				1
Oscillatoria submembranacea Ard. & Straff.	1				1
Nostoc commune Vaucher	7				7
Nostoc muscorum Ag.	5				5
Nostoc sp.	1	1			2
Porphyroshiphon notarisii (Menegh.) Kütz	1				1
Scytonema ocellatum Lyngbye				2	2
Schizothrix calcicola (Ag.) Gom.	23	5	1	3	32

Eighteen species were recovered using the Rotorod sampler. Six of these species belonged to the blue-green algae and 12 species to the green algae. The most common genera recovered by the Rotorod sampler were *Chlorella, Chlamydomonas, Oocystis* and *Schizothrix.*

The identified species obtained by the various collection methods are listed in Tables 1-2.

DISCUSSION

Schlichting (1969) indicated that several workers in the United States and other countries have found a number of diatoms species, yellow greens, and Euglenoids, from the air. The lack of diatoms can not be the fault of an inappropriate culture medium, for other investigators who have reported diatoms from the air used the same basic inorganic medium used in this investigation. Furthermore, our medium was made more favorable to diatoms by the addition of silicon. It seems clear that Florida air over the University Campus from January to August of 1969 contained very low diatom concentrations. The *Euglens* omissions from our collections may well be due to our culture medium which was not enriched with organic

carbon compounds. These missing algal groups also might be due to our algae-poor soil (Smith, 1944; Smith and Ellis, 1943), which is the source of most airborne algae.

Although many workers have reported *Chlorococcum* species to be among the most common of airborne algae, only 3 isolates of 2 species were found in the investigation. The infrequently reported *Schizothrix calcicola* was the most commonly occurring airborne alga in our area.

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